CONSERVATIVE MANAGEMENT OF PARTIAL-THICKNESS SCALD BURNS IN CHILDREN USING CULTURED ALLOGENIC KERATINOCYTE SPRAY: INITIAL EXPERIENCE OF 18 PATIENTS TREATED IN AN OUTPATIENT SETTING

TRAITEMENT CONSERVATEUR DES BRÛLURES INTERMÉDIAIRES PAR EAU BOUILLANTE CHEZ L'ENFANT PAR VAPORISATION DE KÉRATINOCYTES ALLOGÉNIQUES DE CULTURE EN SUSPENSION: ÉTUDE PRÉLIMINAIRE SUR 18 PATIENTS TRAITÉS EN AMBULATOIRE

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SUMMARY. The objective of this study was to describe our clinical experience with the use of cultured allogeneic keratinocyte (CAK) using a simplified cell delivery method in the treatment of pediatric partial-thickness scald burns treated as outpatients in a Burn Unit. An actuator fitted onto a 3ml syringe was used for cell spray. Eighteen patients having active mixed partial-thickness burn wound areas of <10% total body surface area (TBSA), treated between 2017 and 2019, were included in the study. The wounds were managed conservatively with a combination of burn dressings using hydrogels and CAK application. The timing of the CAK application was decided by the treating plastic surgeon based on his clinical judgment and the clinical status of the wound. The primary study endpoints were the number of days and dressing changes required for complete wound reepithelialization. All of the eighteen patients' wounds reepithelialized completely with CAK application, with a mean reepithelialization time of 10.33 (±4.95) days after the application of CAK. The median value for the number of CAK applications and total dressing sessions required to achieve complete healing were 3 and 4 times, respectively. Wounds treated with CAK application between 8-21 days after burn injury required fewer cell application sessions and fewer dressing changes than wounds treated within seven days and after 21 days from the burn injury. None of the patients reported any adverse reaction related to CAK use. The present study suggests that non-extensive mixed partial-thickness scald burn in children can be successfully treated conservatively using CAK as an adjunct in addition to standard dressing in the outpatient setting.

Keywords: allogenic keratinocyte cell therapy, pediatric scald burn, deep partial-thickness burn, outpatient burn treatment

RÉSUMÉ. *Le but de cette étude est de décrire notre expérience clinique avec l'utilisation de kératinocytes allogéniques de culture (KAC), en utilisant une méthode simplifiée d'application cellulaire pour le traitement des brûlures intermédiaires de l'enfant par eau chaude, traitées en ambulatoire dans notre unité de brûlés. Un embout, fixé à une seringue de 3 ml, a été utilisé pour la vaporisation cellulaire. 18 patients présentant des brûlures de profondeur intermédiaire au stade aigu, de surface < 10% de la surface corporelle totale (SCT) et pris en charge entre 2017 et 2019, ont été inclus dans cette étude. Les plaies ont reçu un traitement conservateur combinant des pansements à base d'hydrogel et l'application de KAC. Le moment pour appliquer les KAC a été choisi par le chirurgien plasticien référent en fonction de son appréciation clinique et du stade de la plaie. Les critères principaux d'évaluation de l'étude ont été le nombre de jours et le nombre de réfections de pansements nécessaires à une réépithélialisation complète. Les plaies des 18 patients ont été totalement réépithélialisées par l'application de KAC, avec un délai moyen de 10,33 (+/-4,95) jours après la vaporisation cellulaire. La valeur médiane du nombre d'applications de KAC d'une part et de réfections de pansements d'autre part, nécessaires pour obtenir une cicatrisation complète a été respectivement de trois applications de KAC et de quatre pansements. Les plaies traitées par l'application de KAC dans un délai de 8 à 21 jours après la brûlure ont nécessité moins de séances d'application cellulaire et de pansements que les plaies traitées dans les sept premiers jours ou au-delà du 21e jour après la brûlure. Aucun des patients n'a présenté d'effets secondaires rapportés à l'utilisation de KAC. Cette étude suggère que les brûlures peu étendues intermédiaires par ébouillantement chez l'enfant peuvent être menées à cicatrisation par un traitement conservateur utilisant la vaporisation de KAC en complément de pansements standards en traitement ambulatoire.*

Mots-clés: *kératinocytes allogénique, brûlure de l'enfant par ébouillantement, brûlures intermédiaire, traitement ambulatoire pour brûlés*

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Introduction

Burn is one of the most life-threatening injuries requiring early and rapid wound closure to reduce burn-associated mortality and morbidity.[1] Factors such as burn TBSA, burn depth, patient age and extent of inhalation-associated injury determine the severity of the burn, mode of treatment, and survival prognosis.[2-3] In deep partial-thickness and full-thickness burns, the dermis is damaged extensively, which renders wound healing without scarring difficult; in such circumstances, early excision, followed by wound coverage by split-thickness skin graft, is the gold standard for treating such burn injuries.[4-6] Nonetheless, burn wounds that are deep or have large TBSA take longer to heal and are susceptible to burn wound infections and sepsis. In such cases, the patient's prognosis depends on timely wound excision and closure, limiting wound healing complications such as wound infection and hypergranulation. In contrast, less severe burn injuries in terms of depth and TBSA, commonly treated at outpatient departments or emergency rooms, constitute the majority of burn cases in developed countries. According to previously published data, a large percentage of pediatric burns affecting less than 10% TBSA around the world are due to scalding,[7-11] and the majority of scald burns in pediatric patients are composed of a mix of superficial and severe partial-thickness wounds.[12-13] This sort of mixed depth burn wound complicates accurate identification of burn depth and makes treatment planning for either conservative therapy or aggressive surgical approach difficult for the treating burn surgeon. Additionally, the intrinsic high wound healing capacity of juvenile skin (presence of more adult skin stem cells) adds to the dilemma of whether to treat scald burns conservatively or with early excision and skin grafting, particularly for burns with a burn TBSA of less than 10%. In general, these categories of pediatric patients with 10-15% burn TBSA of mixed burn depths are admitted to a burn unit and monitored for 24-48 hours before being discharged and treated as outpatients with appropriate dressings, unless the majority of the wound is clearly demarcated as deep dermal or full-thickness, necessitating surgical intervention-excision and skin grafting, or there is any other comorbid condition necessitating surgical intervention.[14]

There is still no consensus on the optimal treatment regimen for such mixed depth burns in children.[15] In some therapeutic settings, these sorts of mixed partial-thickness scald burns are aggressively treated with surgical intervention before exact demarcation of deeper portions is established.[16] In such cases, certain intermittent superficial areas with regenerating potential along the wound margin or in the remnant healthy reticular dermis may be excised unnecessarily during tangential excision and skin grafting in such instances, resulting in the requirement for larger skin-autograft and donor site morbidity. In other settings, a conservative approach over surgical intervention is preferred during the acute treatment phase of mixed partial-thickness pediatric burns for various reasons, including the set treatment protocol, physician choice, or resource limitations.[17] However, one major drawback of conservative management is that sometimes isolated pockets of deep partial-thickness burnt areas in a mixed depth wound may take longer to heal. In certain other cases, areas diagnosed immediately after burn injury as superficial partial-thickness may later manifest as or deteriorate into deep partial-thickness or full-thickness wound due to various underlying factors such as inadequate fluid resuscitation, prolonged infection, etc. Delayed reepithelialization extends the treatment length, increases the risk of local and systemic infection, and leads to poor functional and aesthetic outcomes. Availability of an effective and alternative adjunct therapeutic option for expedited wound healing and management of residual deep dermal non-healing burn wounds may help treat these types of mixed burn cases conservatively without any late complication, circumventing the involvement of surgical intervention. One of the conservative management protocols may include initial application of a topical agent acting as mild debriding agent for removal of slough from deep dermal burn wounds and preparation of wound bed, followed by application of a rapid wound-healing agent that can induce migration and proliferation of residual epithelial cells in the wound area and potentiate rapid wound closure. Combining these two agents can achieve expedited wound healing without late-stage complication, and prevent or minimize the need for late-stage skin grafting surgery and the occurrence of donor site morbidity. Clinical application of cultured keratinocytes has been

shown to be effective in the rapid healing of deep and superficial partial-thickness wounds. Thus, cultured keratinocytes can offer a useful therapeutic option for the conservative treatment of such difficult-to-plan pediatric mixed burn wounds. The residual deeper burnt areas that show signs of non-healing can be treated immediately with allogenic cultured keratinocyte cells to achieve rapid reepithelialization. Previously, research groups have used allogeneic as well as autologous cultured keratinocytes as sheet grafts for the treatment of partial-thickness burns.[18-22] However, the preparation of keratinocyte sheet graft requires technical expertise, is time-consuming, and the application of fragile cultured cell sheets is cumbersome. Subsequently, autologous cultured and non-cultured keratinocyte suspension was delivered onto the burn wound in spray form by various spraying tools such as spray nozzle or spray gun.[23-24] The primary advantage of this delivery method is its ability to treat large areas of partial-thickness burns up to 80 times a small skin graft with good clinical outcomes.[25] This cell-spray application method hypothesizes that the pre-confluent cells have a better proliferative capacity than confluent stratified keratinocytes layers in CEA. Hence when the cells are spray applied on the wound, they will continue cell division and proliferate rapidly to cover the wound surface, and will subsequently start differentiating into epithelial layers on confluency and contact inhibition on the wound bed. However, one drawback of the autologous cell-spray suspension spray method is that it still requires a surgical procedure to obtain the patient's skin graft. Thus, autologous source-based keratinocytes cell therapy may not be ideal for outpatient treatment settings. Moreover, extensive studies on the use of keratinocyte cell graft in wound management of burn patients treated in outpatient settings are lacking. The majority of the published clinical studies on the use of cultured keratinocytes have been conducted in inpatient settings. The Burn Unit of Bahrain Defence Force Military Hospital is one of the two major tertiary care centers responsible for treating all types of burns in the island country of Bahrain. As per our past patient statistics, the majority of the burn population treated at our Burn Unit comprises the pediatric population having mixed partial-thickness scald burn less than 10% TBSA.[26] More than 75% of this pediatric patient cohort are treated at the burn unit outpatient burn clinic with customized burn dressings. Encouraged by previous studies showing the efficacy of cultured keratinocytes on expedited wound healing, we initiated a pilot project to develop keratinocyte culture for clinical use in burn wound healing. Human skin samples were acquired from the serologically negative allogeneic source, such as a neonatal foreskin after circumcision surgery, with the informed consent of the patient's legal guardian. We optimized the cell culture protocol using the animal product-free cell culture method. The cultured keratinocyte graft is utilized for multiple clinical indications, including mixed partial-thickness scald burns and non-healing residual burns in adults and children to speed wound healing in inpatient and outpatient settings.

We face the problem of achieving complete reepithelialization of mixed dermal burn wounds employing conservative therapy in our outpatient treatment strategy. Encouraged by previous study results showing comparable wound healing efficiency of allogeneic cells to autologous cells in partial-thickness burns and successful cell delivery using the cell spray delivery method, we developed a modified concept of a combination method combining the two aspects of cell therapy-cultured allogenic cultured keratinocyte (CAK) suspension + spray delivery mode to suit the outpatient setting. The study's primary aim was to assess the usefulness of CAK in achieving expedited wound healing of mixed areas of superficial partial-thickness (SPT) and deep partial-thickness (DPT) pediatric scald burn, which usually takes more than three weeks for complete reepithelialization with conservative management.

Materials and methods

This was a pilot study conducted in the Burn Unit of the Bahrain Defense Force Military Hospital. The study was approved by the Hospital Research and Research Ethics Committee. A total of twenty patients were included in the study as per the study inclusion and exclusion criteria, between October 2017 and October 2019. Two patients were lost to follow-up, the remaining eighteen patients were considered for study analysis. The following patient data were recorded and tabulated in Microsoft Excel for analysis: the patients'

demographic data (age, sex), burn etiology, size of the raw wounds treated with CAK, day of CAK application post-burn, depth of burn wound at the time of CAK application, number of CAK application sessions required to achieve complete reepithelialization, total number of dressing changes required, and the total number of days required after 1st CAK application till complete wound reepithelialization. The study data were anonymized before analysis.

Study inclusion and exclusion criteria

Both male and female pediatric patients between the age of 0.5 years to 16 years, having an active raw area of mixed partial thickness burn wound less than 10% TBSA scald burn at the time of treatment at the outpatient burn clinic, were considered for the study. Patients with full-thickness burn area more than 5% TBSA, infected wound, and any known comorbidity which could impair the normal wound healing process were excluded from the study.

Indications and selection of patients for cell therapy

In our outpatient burn clinic, the standard dressing protocol for treating mixed partial-thickness burn (with the presence of slough in burn wound) in the pediatric population includes two steps. Step one includes regular dressing with a mix of two hydrogels (Amrigel® + Neugel®) at 1:3 ratio to achieve partial debridement of slough, followed by step two, the application of another burn wound healing ointment called moist exposed burn ointment (MEBO) to achieve complete reepithelialization. Some of the patients' wounds were considered for cell therapy by the treating plastic surgeons if they had one of the following two indications: 1) burn wounds with areas of deep partial-thickness (DPT) or mixed areas of SPT+DPT showing signs of impaired wound healing after regular dressing with hydrogel for a minimum of 3-5 days and could take more than three weeks to heal; 2) wounds which were initially diagnosed to be of mixed areas comprising of a majority portion of SPT and tiny pockets of DPT, but then the majority of the SPT wound areas were converted to DPT during treatment due to various complications such as wound necrosis, wound infection, and hypergranulation, etc. and showed delayed healing at three weeks after the injury. Some of those patients were advised for skin grafting, but the patient's parents/legal guardians did not give consent for surgical intervention. Hence they were given cell therapy as an alternative treatment option for expedited wound healing.

Patient informed consent

After the patients were considered eligible for cell therapy as per the inclusion and exclusion criteria mentioned above, their parents/legal guardians were briefed about the cell application and its potential benefits and drawbacks, and written informed consent was obtained from them before the cell application.

Preparation of cultured keratinocytes for clinical use

Cell culture was performed at the shared cell culture facility of the cytogenetics lab in the hospital's in-vitro fertilization (IVF) center. We followed the serum-free and feeder layer-free culture system as previously described, with some modifications.[27] In brief, the neonatal foreskin sample was cut through the middle and flattened. Then, the submucosal layer on the dermal side of the skin was dissected to remove unwanted subdermal parts. The trimmed skin sample was washed thrice for approximately 10 minutes each with Dulbecco's phosphate buffer saline (DPBS, Millipore) with 10% antibiotics solution, which removed the blood clots and topical medicines applied during the surgery. The cleaned skin pieces were cut into 2-3 smaller pieces and incubated in 1.5% dispase II solution (Sigma) overnight at 4-8°C. On the next day, the epidermis was peeled from the dermis with fine forceps and incubated in 10ml of synthetic trypsin solution (TripLE 1x, Gibco) at 37°C for 10-15 minutes till the solution turned turbid. Then, the solution was pipetted in and out further to facilitate the release of cells from the tissue. Fresh 10ml keratinocyte media was added to the TripLE solution, mixed, and filtered through a 40micron strainer (BD). The filtered solution was centrifuged at 1,200 rpm for 10 min. The cell pellet was re-suspended in the Epilife keratinocyte culture medium (Gibco) supplemented with growth supplement S7 (Gibco) and cultured in a T75 cell culture flask (BD). The first media change was done after 48 hours. Subsequent media changes were performed every 2-3 days depending on the color of the medium

and microscopic examination indicating cell growth and level of nutrition exhausted in the culture medium. The cells were sub-cultured at 85-90% confluency till passage six. At each cell passage, 50% of cells were cryopreserved at -196°C in liquid nitrogen in the master cell bank for future cell expansion and clinical use. The cell culture medium of each sub-culture stage was tested for microbial growth immediately before the cryopreservation stage to verify the sterility of the cryopreserved cells.

Preparation of cell suspension on the day of cell application

The cell suspension was prepared 30 minutes before the scheduled time of clinical application and stored at 4-8°C till application on the patient's wound. In brief, the cryopreserved cell vials containing approximately 4-6 million cells/vial were thawed rapidly at 37°C. The number of vials to be thawed was calculated based on the number of cells required (4-6 million cells/50 sq. cm wound). Three ml keratinocyte cell culture medium was added to each vial of thawed cell suspension and centrifuged at 1,200 rpm for 10 min. Simultaneously, viability of the cells was measured in a hemocytometer. After centrifugation, the supernatant was decanted. An appropriate amount of sterile phosphate buffer saline (Millipore) was added to the cell pellet and mixed to achieve homogenous cell suspension of the desired cell concentration. Cell concentration was adjusted to 4-6 million/ml by adding more PBS if required, following which the cell suspension was taken to the burn unit dressing clinic in a cool box, ready for application.

Cell application session and post-application follow-up

Cells were applied topically on the wound during dressing change under the supervision of the treating doctor, wound care nurses, and the skin lab specialist in the outpatient dressing clinic of the burn unit. First, the required dressing materials and the consumables for cell application were arranged at the bedside. The cell pallet brought in the 15ml centrifuge tubes was again mixed in PBS with a sterile pipette and then was loaded into a 3ml syringe. Then the syringe tip was fitted with a sterile cell spray actuator (Coster Pharmaceuticals, Italy) to spray the cells onto the wound. The wound was positioned flat horizontally to ensure the cells did not run down. The wound was cleaned with normal saline and dabbed dry with sterile gauze. Then, the prepared CAK cell suspension was sprayed onto the wound, and the wound was kept undisturbed for 10-15 seconds. Some more cell suspension was sprayed onto the primary dressing (paraffin gauze), and then the primary dressing layer was applied on the wound upside down. Two-three layers of sterile gauze (secondary dressing) were applied on top of the primary dressing layer. The secondary dressing layer was secured with confirming bandage (*Fig. 1*).

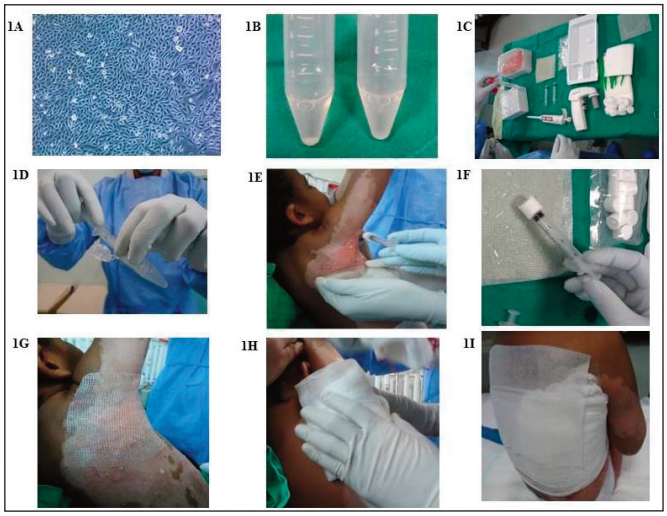

Fig. 1 - Steps for preparation and application of cultured allogeneic keratinocyte (CAK) spray

1A) Microscopic image of cultured keratinocytes (5x magnification); 1B) Cell pallet after centrifugation; 1C) Consumables arrangement for cell therapy at the outpatient setting; 1D) Cells loaded onto a 3ml syringe and fitted with a spray actuator; 1E) Cell spray application on the wound; 1F) Additional cell application on primary dressing (paraffin gauze) layer; 1G) Application of paraffin gauze on wound; 1H) Application of secondary dressing layer (sterile gauze); 1I) The secondary dressing secured with confirming bandage.

Follow-up post cell application

The dressing was inspected every two days after the cell application. Dressing change was not performed unless any soakage was observed on the outer dressing layer. In the case of soakage, the dressing was changed carefully without removing the primary dressing layer. The primary dressing was not disturbed unless there were visible signs of wound laceration and heavy exudation, macerating the primary and secondary dressing layer. In such cases, the primary dressing was removed, and the wound was washed with normal saline and 4% betadine solution, then covered with fresh paraffin gauze and secondary dressing. Those patients who could not come to the burn clinic for follow-up on

day-2 post cell application were followed up on the next day.

On day-4 post previous cell application, both the primary and secondary dressing layers were opened. The paraffin layer was not removed if found stuck to the wound, indicating good healing. The wound was photographed and inspected by the treating doctor. Cell application was repeated if the wound had not completely reepithelialized until complete healing was achieved.

Statistical analysis

The data were entered into excel and later imported to Statistical Package for Social Science (SPSS) version 23.0. The data were non-parametric, Mann-Whitney U test was used to compare two groups, and Kurskal-Wallis H test was used to compare more than two groups. A Chi-Square or Fisher's exact test was used on discrete variables. A p-value less than 0.05 was considered statistically significant.

Results

A total of 18 pediatric patients with mixed partial-thickness scald burn wounds who were treated with the cell therapy protocol at different time points during the treatment and fulfilled the study inclusion criteria were included in the study for analysis. Wounds on all body areas (head, trunk, upper limb and lower limb) were included in the study. Out of the total 18 patients, 10 (55.6%) were female, and the remaining eight (44.4%) were females with a mean age of 28.61±33.97 months and median (interquartile range) age of 19 (14). *Table I* describes the patients' demography and the clinical characteristics of these patients' wounds. The mean and median (IQR) size of the wound treated with CAK was 114.83±113.59 sq. cm and 87.5 (115) sq. cm, respectively. Out of the total 18 patients with mixed partial-thickness burn, 38.89% (7) patients' wounds had an almost equal proportion of superficial partial-thickness (SPT) and deep partial-thickness (DPT). Those wounds were subcategorized as SPT+DPT wounds, and the remaining 61.11% (11) patient wounds were categorized as DPT (wounds with 75% or more area of deep partial-thickness wound and the rest as superficial partial-thickness). The wounds were treated with cell therapy at various periods ranging from day-5 to day-39 post-burn. Hence the time of cell application was divided into three groups: 0-7 days, 8-21 days, and beyond 21 days. Five patients were treated within 0-7 days, seven patients within 8-21 days, and the remaining six patients after 21 days of burn injury. All 18 patients (100%) achieved complete wound healing. The mean and median wound healing time after application of cell therapy for all 18 patients was 10.33(±4.95) days and 9(5) days, respectively. We compared the healing time and other treatment parameters between the two subgroups: (SPT+DPT) and DPT wounds as described in *Table II*. A chi-Square or Fisher's exact test was used to analyze the association between demographic variables. There was no association between any discrete variables such as age and location of the wound with the depth of the burn. Mann-Whitney U test was used to compare the continuous variables such as treatment lag, the number of cell application sessions, the total number of dressings, etc. (*Table II*); however, there was no statistically significant difference between the variables and depth of the burn. The median(IQR) wound healing time of seven patients with SPT+DPT burn wound, and eleven patients with DPT burn wounds were similar, at 9(8) and 9(6) days, respectively. Then we investigated if the timing of cell application affects wound healing irrespective of wound depth (*Table III*). We found that the median (IQR) healing time for wounds treated after 21 days of burn injury was higher at 13(7.8) days compared to 9(6.5) days for wounds treated within seven days, and 9(3) days for wounds treated between 8-21 days. This could be because the deep-dermal, non-healing and chronic condition of DPT wounds delayed healing. The study analysis also showed that although the median healing time of wounds treated between 8-21 days and 0-7 days are the same (9 days), there was a statistically significant difference observed in the requirement of secondary dressing changes between the three groups of time of cell application. The median value was the highest, at 2(1.5) times in the >21 days groups. The number of secondary wounds treated between 8-21 days required fewer secondary dressing changes and cell application sessions than wounds treated within 0-7 days (*Table III*). This lesser number of dressing changes could be because of reduced exudates from

Table I - Demographic and clinical characteristics of patients

Variable	Frequency	Percentage (%)
Gender		
Female	10	55.6
Male	8	44.4
Age (months)	Mean±SD 28.61±33.97 Median(IQR) 19(14)	
Size of wound treated (cm^2)	Mean±SD 114.833±113.59 Median(IQR) 87.5(115)	
Treatment lag (gap between burn and 1st cell application)	Mean±SD 17.44±11.39 Median(IQR) 13.5(20.8)	
No. of cell application sessions	Mean±SD 2.89±1.37 Median(IQR) 3(1.3)	
No. of secondary dressings required	Mean±SD 1.28±0.96 Median(IQR) 1(1)	
Total no. of dressings required	Mean±SD 4.22±2.41 Median(IQR) 4(2)	
Total no. of days taken for complete healing after cell application	Mean±SD 10.33±4.95 Median(IQR) 9(5)	
Total no. of days taken for complete healing from the day of burn injury	Mean±SD 23.6±8.6 Median(IQR) 21.5(13.25)	
Wound depth		
DPT	11	61.11
SPT+DPT	7	38.89
Total	18	100
Location of wound (H, T, U, L)		
H	1	5.56
L	5	27.78
T	7	38.89
U	5	27.78
Wound status at the end of treatment		
Complete healing	18	100

H = head, L = lower limb, T = trunk, U = upper limb

Table II - Comparison between two groups of burn wounds as per the depth of the wound

	Type of burn		
	SPT+DPT	**DPT**	**P Value**
Age (months)			
10-24	6 (85.7%)	8 (72.75%)	0.748
25-48	0	2 (18.2%)	
>48 months	1 (14.3%)	1 (9.1%)	
Location of wound			
H	0	1 (9.1%)	
L	1 (14.3%)	4 (36.4%)	0.692
T	4 (57.1%)	3 (27.3%)	
U	2 (28.6%)	3 (27.3%)	
Treatment lag (days to 1st cell application) (median (IQR))	21.00 (26.0)	12.0 (20.0)	0.596
No. of cell applications (median (IQR))	3 (2)	3 (2)	0.425
No. of secondary dressings (median (IQR))	1 (1)	1 (1)	0.86
Total no. of dressings done (median (IQR))	4 (3)	4 (3)	0.596
Days to heal (D2) (median (IQR))	9 (8)	9 (6)	0.425

*p<0.05 is considered as statistically significant

Table III - Comparison between the time of cell applications (treatment lag) and wound healing

	Time of cell application			P Value
	0-7 days	**8-21 days**	**>21 days**	
Size of wound treated with cells (sq.cm)	120 (295)	100 (175)	50 (62)	0.278
Treatment lag (days to 1st cell application)	**6 (2)**	**13 (6)**	**31.5 (10.8)**	**0.001***
No. of cell applications	3 (2)	2 (1.0)	3.5 (2)	0.122
No. of secondary dressings	**1 (1.5)**	**1(1.0)**	**2 (1.5)**	**0.043***
Total no. of dressings done	4 (3.5)	3 (2)	5.5 (3.8)	0.054
Days to heal (D2)	9 (6.5)	9 (3)	13 (7.8)	0.121

*p<0.05 is considered as statistically significant

	Cell application
Treatment lag	0-7 vs 8-21 ; p=0.004
	0-7 vs >21 ; p=0.006
	8-21 vs >21 ; p=0.001*
No. of secondary dressings	0-7 vs 8-21 ; p=0.343
	0-7 vs >21 ; p=0.329
	8-21 vs >21 ; p=0.022*

burn wounds after one week of burn injury. This result suggests that the time after one week and before three weeks of the burn injury can be considered the best time for cell therapy. The representative images of four patients' wound status before and after cell application are given in *Fig. 2.* We compared our study data (total wound healing time from the date of burn) with a historical comparable cohort data serving as a control group from the previously published study by delli Santi et al.[28] involving similar patient demographies and wound conditions. In that pilot study involving five pediatric patients having mixed partial-thickness scald burn, the wounds were treated conservatively using a combination of enzymatic debridement (Nexobrid™) and application of a biosynthetic cellulose matrix dressing (Epiprotect®). The study data showed the mean total wound healing time was 31.2±3.5 days after the burn injury. In the present study, the mean total wound healing time was 23.6±8.6 days after burn injury. The comparative data from the two studies suggest that the application of CAK could have contributed to the reduction in the total wound healing time by one week as compared to the standard treatment. This indicates the expedited wound healing efficacy of cultured allogenic keratinocyte spray compared to advanced dressings in the conservative management of pediatric scald burns.

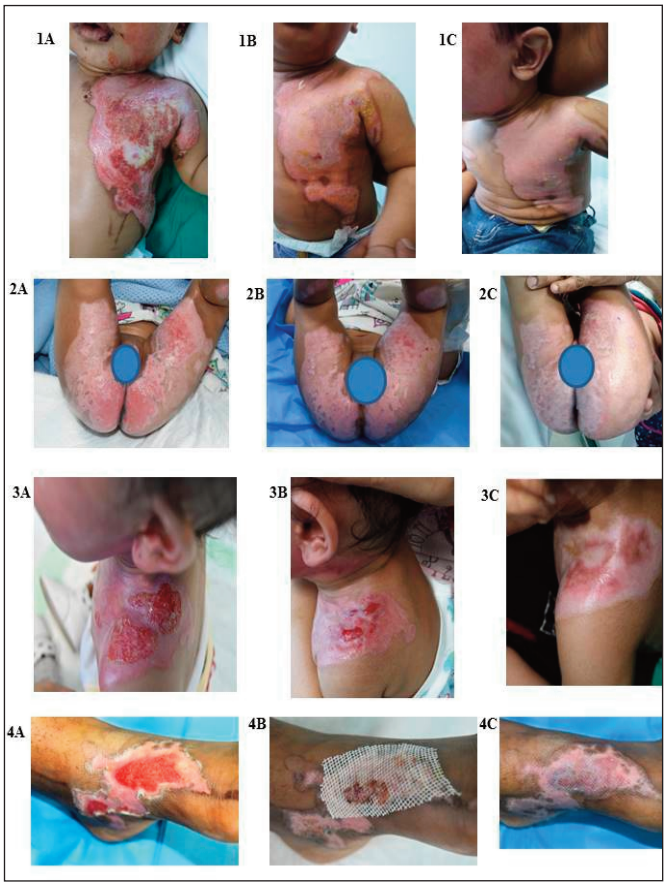

Fig. 2 - Representative photographs of the burn wounds (pre and post-op) of four patients treated with cultured allogenic keratinocyte spray
Numbers 1-6 denote patient serial number. "A" denotes wound status at the start of CAK application. "B" denotes wound status at the middle of CAK application. "C" denotes wound status (complete healing) at the end of treatment.

Discussion

This study studied the feasibility and efficacy of successfully treating mixed areas of partial-thickness scald wounds with CAK suspension spray in children in an outpatient setting. Similar to other studies, we observed that most scald burn victims were toddlers in the age group 0-5 years.[11-14] No surgical debridement was required in any patients for wound bed preparation before cell application. The cells were spray suspended onto the wound by simply wiping the wound bed with sterile saline-soaked gauze. This was feasible as the wound beds of mixed partial-thickness burn were soft and slimy and started to slough off after 3-4 daily dressings with hydrogels. Sloughing of burn eschar revealed a patchy wound bed with pockets of healthy granulation tissue, which might have assisted in the applied cells' adhesion and subsequent expansion. Thus, avoidance of mechanical debriding methods might have helped retain vital tissues in mixed thickness burn wounds, which would have expedited cell growth by secreting essential growth factors.[29-30] We repeated the keratinocyte application every four days to compensate for the partial loss of cells in suspension during subsequent change of primary dressing layer (paraffin gauze) in a majority of the patients to achieve rapid healing and minimize the risk of infection. We observed complete reepithelialization in all wounds, irrespective of the wound size, location of the wounds, and time of the start of CAK application.

Cultured epithelial autografts (CEA) have been used successfully since 1980 for large full-thickness burn wounds.[19,20] The main advantage of this method is the availability of a large skin area from a relatively small biopsy sample of healthy skin. The major disadvantage is the long time required for generating the material, which might delay the treatment of extensive deep burns, leading to life-threatening infections.[31,32] As an alternative, the use of allogeneic skin grafts (allografts) has also been in clinical use for treating deep and partial thickness burn wounds,[21,22] albeit with contrasting results.[33] Opinions vary on the extent of viable epithelial cells remaining at the graft site after removing the paraffin jelly-based dressing. Nonetheless, this method is rapid, and cells can be frozen for long periods in a skin cell bank, ensuring the cells' ready availability under all circumstances. Indeed, cultured keratinocyte allografts have been used successfully to treat first and second-degree deep burns.[29,30] The time of recovery is generally 1-2 weeks, depending on the thickness and size of the wound. Recently various cell delivery methods are evolving to achieve better clinical outcomes. Epidermal cells are also cultured on bio-compatible scaffolds for better handling and proliferation in the wound. Skin-derived precursor cells were differentiated into keratinocytes on collagen-chitosan scaffolds, promoting wound healing in a rat model of burn wounds.[34] Similarly, autologous epidermal and dermal cells isolated and grown on elastin-collagen scaffolds achieved excellent wound healing when transplanted in a patient with severe burn wounds.[35] Currently, autologous non-cultured keratinocyte cell sprays are used for the treatment of superficial as well as deep-partial thickness burns.[36,37] This is especially useful because of the less time required to generate these cells, simple logistics of use in the outpatient department, and comparable clinical outcomes to cultured cell application. Products such as ReCell (using cell spray technology) have also been used as an effective adjunct in combination with STSG to achieve better functional and cosmetic outcomes.[37] However, non-cultured cell spray can be used on a limited area and is not ideal for treating larger burn surfaces. Cultured and non-cultured epithelial autografts in liquid form also increase the take rate of wide meshed grafts in massive burns.[38,39]

Treating pediatric burn cases is challenging because skin available for replacement is minimal, donor area could expand, and subsequent hypertrophic scar and contracture could become more prominent along with physical growth. Yanaga et al.[40] used cryopreserved epidermal cell allografts to treat deep partial-thickness and split-thickness skin donor sites in 55 children and achieved satisfactory clinical results. They achieved early closure of wounds and suppression of scar formation. Rab et al.[41] performed a study on pediatric dermal scald burns using both autologous skin graft and allogeneic cell-cultured grafts. They observed that the children who received the allogeneic cell-cultured grafts achieved complete reepithelialization in 90% of the treated patients within 12 days with no secondary grafting require-

ment and reduced blood transfusion requirement. Mcheik et al.[42] demonstrated the potential of foreskin-derived keratinocytes as autologous cell grafts. These cells formed structured multilayered epithelium in vitro, highlighting the potential of foreskin tissue for autograft application in boys. Here, for the first time, we report the advantage of using foreskin-derived keratinocyte spray without any surgical wound debridement in pediatric scald burn healing in an outpatient setting in Bahrain. This is in contrast to the method used by Gerlach et al.,[43] who used collagenase treatment for enzymatic wound debridement before using an autologous keratinocyte spray in an ambulatory setting in only one patient. This is probably the first study to investigate the wound healing efficacy of cultured allogeneic keratinocytes in suspension spray form for burn, applied in an outpatient setting. Our method is especially beneficial in the family setting of Gulf countries, where large families with closely spaced children are preferred. Hence, most parents prefer treatment as an outpatient so that the mother can also attend to other siblings of the patient at home. Reduction in the treatment time enables parents with multiple young children to manage both hospital visits and home without missing work.

Moreover, this technique is easy to execute and not labor-intensive. Since culturing of cell sheets or biocompatible scaffolds is not required, the cost of production is also relatively less than cultured cell sheets. Finally, the cultured cell suspension is easy to transport and can be used in smaller centers (with low human resources and expertise) or outpatient settings, thereby circumventing the need for operation theatres, while achieving results similar to those obtained with allogeneic epidermal sheets.[44,45] We observed that patients treated within three weeks of acquiring the burn required a fewer (1-4) number of cell applications than those treated after three weeks (2-7), indicating that expedited wound healing was obtained with earlier therapeutic intervention. This result agrees with the observations of Cubison et al.[46] and Chipp et al.,[47] who found that the probability of developing hypertrophic scars diminishes, and chances of healing increase if pediatric scald burns are treated within 21 days of the burn injury. A recent study showed that wound dressing materials variably affect the attachment, proliferation and expansion of autologous sprayed primary keratinocytes, which subsequently affects wound healing.[48] However, we did not observe any adverse effect of paraffin dressing on wound healing in our study.

However, our study is not without limitations. Since the wound sizes were small, we could not demarcate a control and treated area within the same wound for comparison. In addition, we encountered problems related to the running-off of the sprayed liquid on non-flat body surfaces such as the neck and back, which might affect the efficacy of the treatment. For such cases, specific postures have to be designed to ensure flattening of the target surfaces during the therapy to bypass this problem. Finally, the sample size used in this study is small, and studies with a larger patient pool should be conducted in the future to validate the efficacy of this treatment protocol further.

Conclusions

Cultured keratinocytes are clinically effective in achieving rapid healing of deep and superficial partial-thickness wounds. Thus it is a useful therapeutic option for conservatively treating pediatric mixed burn wounds. Keratinocyte sheets with fixed sizes are more suitable for covering large wounds. The cell suspension method gives flexibility in preparing the appropriate amount of cell suspension as per the wound dimension. Allogeneic cell source ensured instant availability without any waiting time. The use of the actuator helped deliver the cells as fine particles and helped in sticking to the wound instantly. The initial dressing with hydrogels worked well for removing minimal to moderate soft slough and wound bed preparation for cell therapy. By avoiding debridement, vital tissues were preserved, and the applied cells got attached to those pockets of the clean raw area and induced the host cells to divide and proliferate to close the wound. Our results suggest that allogeneic cell spray application in a burn outpatient setting is a feasible and effective treatment option for the rapid healing of partial-thickness scald burn wounds.

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