Research Square

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Sprayed cultured autologous keratinocytes in the treatment of severe burns: a retrospective matched cohort study

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Research note

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Abstract

Objective: Cultured autologous keratinocytes were used to treat burns since 1981 with many challenges and variable clinical outcome. Our aim was to compare the duration of hospital stay between burned patients received skin grafts with and without cultured autologous keratinocytes, in a retrospective cohort study. Multivariable regression was used to analyse associations between duration of hospital stay and treatment adjusted for age, mortality, size, and depth of the burn. Then, we investigated the differentiation of human stem cell line to keratinocyte-like cells as a future perspective.

Results : The median hospital stay in the group given cultured autologous keratinocytes (n=12) was 70 days (10 th – 90 th centile 50-141) and in the matched group 62 days (2 -196) (n=43), p=0.25. The multivariable regression analysis showed a coefficient of 17.36 (95 % CI -17.69 to 52.40), p= 0.32, for hospital stay in the treatment group, compared with the matched group. Our results showed no difference in the duration of hospital stay between the two groups. Therefore, further evaluation for this treatment is needed. Stem cells showed enhanced proliferation and differentiation when cultured on glass surface rather than the classical tissue culture plastic and should be considered for burn management research.

Introduction

The golden standard for the treatment of deep burns is excision followed by coverage with an autograft, which is limited by the availability of donor sites. Thus, the development of novel techniques to create bioengineered skin is highly needed in clinical practice [1, 2]. In Linköping Burn Centre, we have been using cultured autologous keratinocytes (CAK) in addition to split thickness for patients with middle-sized burns [3-6]. Keratinocyte were delivered as single cells by aerosol-based spraying on the wound, which resulted in quicker epithelialisation [3, 7]. When CAK was combined with split thickness skin grafts or Meek micro grafts, healing time was shorter [6, 8-10]. One of the possible drawbacks of the widely-meshed autografts, apart from their delayed healing, is the risk of scarring [11]. With the current technical development, the question still remains: does the treatment of burns with CAK accelerate healing time and shorten the duration of hospital stay? This study aimed at comparing the duration of hospital stay between burn patients treated with autologous skin grafting with and without CAK.

Patients And Methods

This study was a retrospective cohort with matched groups, included patients admitted with burns and treated with CAK at the Burn Centre in Linköping between 2012 and 2015. Twelve patients were included in the study and matched individually by age, size and depth of the burn, with a total of 43 control patients admitted to our burn centre during 2008-2015. Data were retrieved from the local database [12] and the hospital medical records. All patients had staged excisions before final autografting with split thickness skin grafts [13]. The control group was treated with meshed autografts on deep burns. The same routine was used for the treatment group with the exception of the extra use of CAK. Full thickness skin biopsy specimens (2 x 2 cm up to 6 x 3 cm) were harvested with a scalpel under sterile conditions as near as possible to the day of admission. The biopsies were cut into small pieces and incubated overnight in dispase as 13.7 mg/ml of Dulbecco's Modified Eagle Medium (DMEM). Then, the epidermis was separated, incubated with trypsin and keratinocytes were obtained by centrifugation. The cells were cultured in a serum-free keratinocyte medium (with L-glutamine) with 25 µg/ml of bovine pituitary extract and 1 ng/ml of epidermal growth factor. The sterile environment was checked for microbial growth one week before the planned treatment and on the day of treatment. When the cells became 70%–75% confluent, they were sub-cultured. A maximum of three passages were allowed.

On the day of transfer, the cells were prepared as 1 x 10^6 viable cells/ ml of DMEM. Following debridement, the cell suspension was mixed with fibrin glue and sprayed on the surface of the wound either alone, with a Meek micrograft, or a widely-meshed autograft that would expand from 3:1 to 6:1. The autograft was harvested at 8-12/1000 inches using a pneumatic dermatome and meshed using a skin graft mesher [10, 14]. Cells were sprayed on the surface of the wound and then the micrograft was applied. The meshed autografts were applied in the reverse order; i.e. the autograft was applied first and fixed with staples or fibrin glue, and the cell suspension was then sprayed on top of the graft. Each sprayed area was photographed when the wound was first taken down (5 -11 days after treatment) and 2, 3 and 4 weeks after treatment. The “take” rate was evaluated by two independent surgeons through digital imaging. The scar was assessed three months after injury with the Vancouver scar scale (0 = normal and 13 = the worst scar possible) [15]. STATA (STATA

version 12.0, Stata Corp. LP College Station, TX, USA) was used for statistical analysis. Descriptive data are given as median (10-90 centiles). Probabilities of less than 0.05 were accepted as significant. To detect any significant differences in the duration of hospital stay between the two groups, we used the two sample Wilcoxon rank-sum test and Fisher's exact test. A multivariable regression was used to analyse the significance of all the associations between duration of hospital stay and the study groups adjusted for percentage total body surface area burned (TBSA %), age, loss of skin thickness %, and mortality.
Human immortalized bone marrow stromal cells (iMSC; ABM, Canada) were cultured in minimum essential eagle's media with 10% fetal bovine serum, 1% antibiotics and dexamethasone as 10^{-6} M, for seven days with media change every other day [16]. Cells were cultured on two different surfaces, tissue culture plastic and glass, as the latter can modulate cell adhesion that is essential for keratinocyte differentiation [17]. The media was supplemented with either 10^{-6} M retinoic acid and 100 ng/ml epidermal growth factor (EGF). After one week, the media was removed and cells were prepared as described before [18]. The cells were stained with anti-cytokeratin 14 antibody (Abcam, United Kingdom), according to the manufacturer protocol.

Results

Patient characteristics were summarized in Table 1. Skin biopsy was obtained on median 3 (1-18) days following admission, and required 29 (18-36) days to obtain enough keratinocytes to cover 5% of the body surface area with about 0.63 $x10^6$ cells per TBSA % (approximately 4000 cells/ cm^2). Eight patients were treated with cells once, two patients twice, one three times, and one four times. Cells were applied to different areas on the different occasions. The Median "take" rate of the graft was 80% one week after treatment.

Table 1. Details of the patients by treatment group

	CAK	Control	p value
Patients	12	43	
Sex (male)	8 (67)	32 (74)	1.00
Age (years)	43 (20-62)	45 (24-73)	0.71
Deaths	2 (17)	5 (12)	0.64
TBSA%	49 (24-81)	48 (21-72)	0.41
Full thickness burns BSA%	18 (0-54)	28 (2-49)	0.57
Deep dermal burns BSA%	17 (2-36)	9 (2-29)	0.23
Superficial dermal burns BSA%	1 (0-10.5)	0.5 (0-19)	0.88
Hospital stay, days	70 (50-141)	62 (2-196)	0.25
Burn type (thermal)	12 (100)	40 (93)	1.00

Scars on the areas treated with CAK were assessed in 9 patients as one who did not attend for follow up and two patients died. Median Vancouver score was 8 (6-10). Scars were assessed as hyperpigmented in eight patients and hypo pigmented in one. Vascularity was assessed as “red” in five patients and in the other four varied from normal to “purple”. In six of the patients the scar was firm but pliable, while the remainder were recorded as yielding, banding, or contracted. No scar was of normal height, but eight were assessed as < 2 mm and one between 2 and 5 mm.

There was no difference in the duration of hospital stay between the groups. The multivariable regression model showed a higher coefficient for the treatment group, together with a wide variation, so the result was not significant (p value= 0.32). The only factors that affected duration of hospital stay were death and the size of full thickness burns (Table 2).

Table 2. Multivariable regression for effect on duration of hospital stay.

	Coefficient	p value	95 % CI
Sex, male	- 2.27	0.89	(-34.74 to 30.19)
Age, years	- 0.34	0.45	(-1.21 to 0.54)
Mortality	- 74.41	0. 005	(-125.14 to -23.67)
TBSA%	0.60	0.18	(-0.28 to 1.47)
Full thickness burns BSA%	1.57	<0.001	(0.77 to 2.38)
CAK*	17.36	0.32	(-17.69 to 52.40)

Model adjusted R^2 0.35 <0.001, n = 55

*the matched group is reference

iMSC cultured on glass had higher density than those cultured on the classical tissue culture plastic, in spite of the equal seeding density. In addition, glass enhanced the expression of cytokeratin 14 in all studied culture conditions. While dexamethasone with and without epidermal growth factor had higher cell density, retinoic acid enhanced the expression of the studied marker per cell (Figure 1).

Discussion

In agreement with previous studies, no statistical difference was observed in the duration of hospital based on receiving CAK following the adjustment for TBSA% [19-21]. In a retrospective study involved 20 children who survived massive burns, the use of CAK, cultured as sheets was associated with longer hospital stay (128 compared to 89 days in controls) [22]. The median duration of hospital stay in our treatment group was 1.8 days (1.2- 2.8)/ TBSA%, which is similar to that of other burn centres that treat adult patients with moderate -sized burns who require operation [23]. Among patients who have been treated with CAK, hospital stay has been reported to be between 0.8- 5 days/TBSA%, usually about 1.5 days/TBSA%, which is similar to our findings [6, 10, 19, 20, 22, 24-27].

Other evaluated parameters showed no difference between the two study groups, which was not in agreement with previous studies. For example, more survivors in the group treated with CAK was reported in an earlier paper [19]. Our “take” rate was lower than [28], similar to [6], or better than [29] which used the same techniques. Scarring was assessed after three months, when the median Vancouver score was 8, which is “severe” compared with others who have reported scores between 2.4 [9] and 5.8 [29]. The assessments were made later in these studies of more mature scars, which may explain why they had a better scoring than our patients [11, 30]. The method of using CAK has been reported to reduce the need for donor sites as well as accelerating the healing of donor sites of split thickness skin grafts. When it has been used for deep burns, several limitations were described, such as the time needed to culture cells, the fragility of the epidermal layer, the lack of dermis that results in the formation of blisters, prolonged hospital stay, severe scarring and related expenses [24, 27, 31]. Our culture time was somehow longer than what have been reported before (12 - 21 days) [9, 28, 29, 32]. Alternatives to the classical CAK, tissue-engineered products that combine keratinocytes with other cell types, micro barriers, or scaffolds could be considered [22, 31, 33-37].

In another direction, we investigated the possibility of the differentiation of a mesenchymal stem cell line into keratinocyte-like cell with different culturing conditions. Cytokeratin 14 is an important protein that found mainly in the keratinocytes of the basal layer of epidermis, which is responsible for giving the rest of the epidermal cells. This protein is important for the cell protection as well as their proliferative capacity [38]. Short term culture for 7 days was associated with the expression of this marker under different culturing conditions. Glass, as an available and cheap culture material was associated with enhanced proliferative and differentiation capacity of iMSC. The possible explanation was providing an adhesion and migration surface of iMSC during their culture and differentiation. Such effect has been shown before in stem cell differentiation into the osteogenic lineage with various biomaterials [39]. These results would provide a new direction in cell-based therapy for our burn victims.

Limitations

The retrospective design of the study is a limitation. Patients treated with CAK were those with large wounds that had not healed at the time when the cell culture was ready. Another important factor is the possible heterogeneity of the isolated cells. Melanocytes, fibroblasts, angioblasts as well as epidermal stem cells might have been included during the isolation process. In additions, keratinocytes can un-differentiate in the culture system into earlier precursors [40, 41]. The one centre approach with results generated from a relatively small group is another limitation that makes it difficult to generalise, although this sample is a usual trend in similar studies [29].

Based on the results we will also start a new program for culturing patient's autologous stem cells and their differentiation into keratinocyte like cells. This step will need to follow the strict regulations of advanced therapeutic medical products in order to be applied clinically.

Declarations

Ethics approval and consent to participate:

Ethical approval was obtained from the Regional Ethics Review Board in Linköping (No. 2013\341-31).

Consent to publish

Not applicable

Availability of data and materials

All related data are included in the manuscript

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

MK and IS were involved in following up the patients, recording and analysing the clinical data. PO, JT, FS and ME were involved in patient recruitment, applying the cells and surgical care. LA was responsible for keratinocyte isolation and expansion. SF, AK, DP and AE were involved in stem cells related experiment. MK, IS, AE and ME were involved in the data analysis and final manuscript writing.

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List of Abbreviation

Cultured autologous keratinocytes	CAK
Dulbecco's Modified Eagle Medium	DMEM
Epidermal growth factor	EGF
Human immortalized bone marrow stromal cells	iMSC
Total body surface area burned	TBSA

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Figures

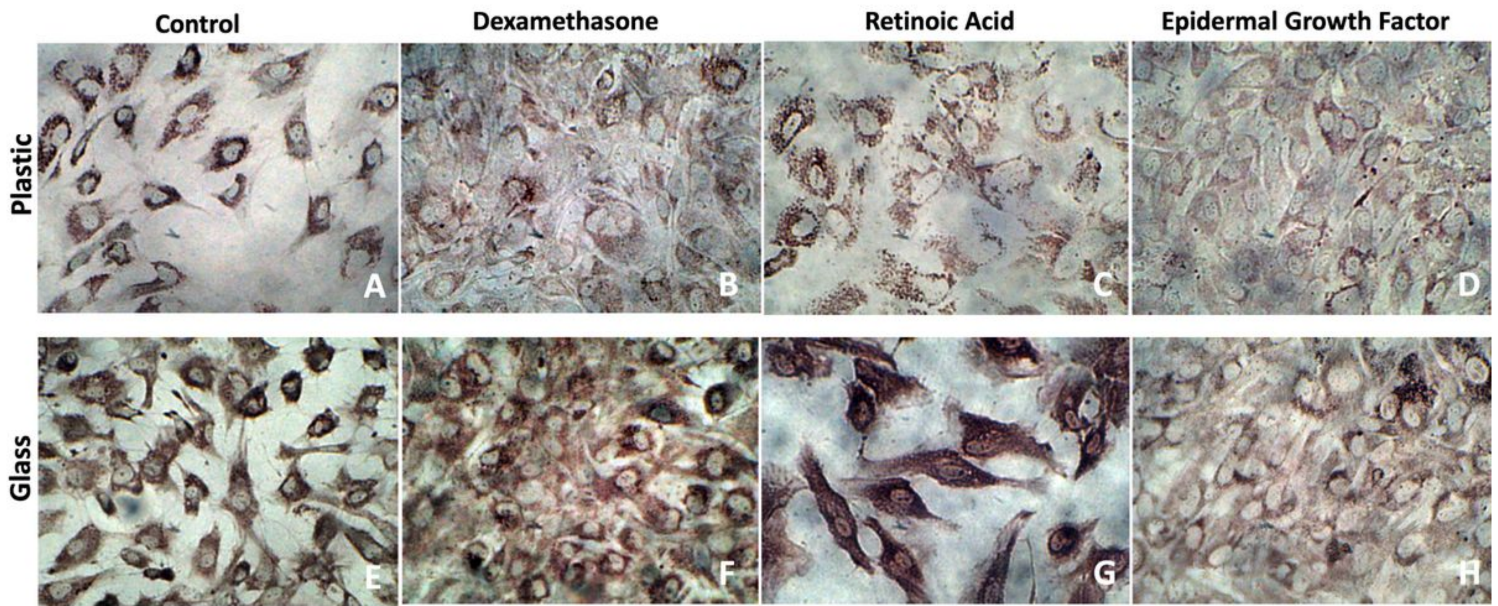

Figure 1

Cytokeratin immunostaining for cytokeratin 14 in iMSC cultured on plastic (A-D) and glass (E-H) with different media additives. Cells cultured on glass showed higher density than those cultured on plastic with enhanced expression of the protein. All the images were photographed at 40X.