Case report

Autologous skin cell spray-transplantation for a deep dermal burn patient in an ambulant treatment room setting

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1. Introduction

While superficial partial-thickness burn wounds are treated conservatively and split-thickness skin grafting (STSG), with or without mesh expansion, is unequivocally indicated for full-thickness burns, the indication for STSG to a deep partial-thickness burn wound remains obscure, if not controversial. Delayed healing with its associated complications (i.e. hypertrophic scarring, contracture, infection, and unsatisfactory psycho-social adjustment) [1] must be balanced against overgrafting, particularly when the hands and face are involved [2]. Delays in deep-dermal wound healing can typically be appreciated by week 2 of conservative management. Unfortunately, this is a particularly problematic stage for traditional skin grafting.

Autologous single-cell skin grafting as an alternative treatment in this situation has been described previously by Wood and co-workers [3–5]. Much like a traditional STSG, autologous skin-cell transplantation is based on removing healthy skin, at a superficial dermal depth, using a dermatome, from a non-prominent, unburned area of the body in an operative setting. Skin cell isolation is performed immediately after harvest and spray-grafting to the burn site is applied during the same operative session. In comparing skin cell spray-grafting with STSG in the deep dermal wound, Gravante et al. found skin cell grafting to be well tolerated and similar in results to STSG [6]. The advantage of a reduced donor skin area for spray-grafting, however, is associated with a non-desirable prolonged general anesthesia time required for the cell isolation.

Cell spray-grafting that could be enabled in an outpatient treatment room setting without general anesthesia would present advantages. We have modified this technique for patients in whom a late stage STSG may be problematic but for whom conservative treatments have been unsatisfactory. In contrast to Wood et al., we employ 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 enables to separate dermis from epidermis and this allows 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. We report our modified method and our 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.

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doi:10.1016/j.burns.2011.01.022
2. Methods

2.1. Patient

Autologous skin-cell spray transplantation was performed at our center on a 43 years old male patient in 2009. The Institutional Review Board, through its Technology and Innovative Practice Assessment Committee, approved the procedure. The patient suffered a gasoline flame burn to his face, right shoulder, arm and elbow. The total body surface area (TBSA) was 7%.

2.2. Initial wound treatment after trauma

The patient presented to our burn center 5 days after the injury. He was initially treated in an outside facility with antibiotic ointment to the face and silver sulfadiazine cream on the arm and shoulder, which he applied daily at home. He was referred to our facility by his primary care physician for concerns of delayed wound healing of the arm and shoulder. On our initial evaluation he was diagnosed to have a deep partial-thickness burn to the shoulder and arm with an inflammatory eschar. While the wounds on face and neck were relatively superficial, the area of concern on the shoulder, elbow and arm were moderate deep partial-thickness and measured 5% TBSA. No tangential wound excision or escharotomy was performed; he did, however, undergo a mild mechanical debridement and began daily local wound care using collagenase (Santyl®, Healthpoint, Ltd., San Antonio, TX) ointment and polysporin powder (Johnson and Johnson CCI, Skillman, NJ) along with daily inspection in our hydrotherapy unit. His eschar began to lift; however, he still did not have significant reepithelialization of the wound and was thus considered a candidate for single-cell spray application as an alternative to STSG. The patient is physically active in his profession and we were concerned that without some epithelial grafting, a hypertrophic scar or contractive contraction could develop across the elbow joint. Informed consent was obtained and alternative treatments explained. We explained to the patient that the decision would have no impact on potential alternative treatment. Pre-existing local and systemic infections, hypersensitivity to trypsin or other enzymatic wound treatments and the risks associated with anesthesia were excluded.

2.3. Cell spray-transplantation

The entire procedure was performed in an outpatient treatment room. On post-burn day #10 the patient underwent single-cell transplantation. His right hip supplied the donor skin. A 2 cm × 2 cm graft was taken to a depth of 0.008 in using a Weck® blade (Teleflex Medical, Research Triangle Park, NC) after chlorhexidine preparation (Hibiclens® MÖlnlycke Health Care US, LLC, Norcross, GA) and were disposable. During donor skin processing, and prior to transplantation, the burn wound was prepared using Hibiclens® solution and gentle mechanical debridement until bleeding spots occurred. To reduce pain, 400 mcg of fentanyl (Actiq®, Cephalon, Inc., Frazer, PA) was administration orally. Hemostasis was performed with regular gauze.

4. Wound treatment and follow-up

Following cell spray-grafting the wound was dressed in Adaptic® (Johnson and Johnson Medical Limited, Gargrave, UK) and wrapped in dry gauze (Kerlix®, Tyco Healthcare Group LP, Mansfield, MA). For clinical follow-up evaluation to assess burn scars we used the Vancouver Scar Scale [8].

2.5. Skin cell isolation

The biopsy treatment includes a modification of a previously described [7] two-enzyme approach of tissue digestion using dispase and trypsin, and cell washing using conventional clinical cell centrifugation. The following steps for autologous skin-cell spray transplantation were performed; this procedure required approximately 70 min:

A) The donor area biopsy is performed in a routine way, as known from split mesh skin grafting. The biopsy is transferred into a sterile disposable 100 mm Petri-dish (BD Biosciences, Bedford, USA) and is cut into 3 mm × 4 mm pieces by the surgeon with a surgical scalpel. We took care that the specimen would not dry out.

B) The cell separation, performed by a specialized laboratory technician, involves

1) initial separation of dermis and epidermis for 25–40 min in a 2.5 unit/ml Dispase II (#4942078, Roche Diagnostics, Mannheim, Germany) containing Ringer’s lactate solution (Lactated Ringer’s Injection USP 1000 ml bag, Baxter, Deerfield, IL) at 37 °C in Petri-dishes (BD);

2) mechanical separation of dermis and epidermis of each skin piece using forceps and scalpel, performed in a dry Petri-dish (the specimen must not dry out), the dermis parts are not used further;

3) transferring the epidermal pieces in a 15 ml Falcon tube (BD) with Ringer’s lactate solution, to be washed from the enzyme;

4) placing the epidermal pieces into a single cell suspension for 10–15 min in a 5 ml 0.05% trypsin/0.02% EDTA containing solution, at 37 °C in 15 ml Falcon tubes to allow isolation from epidermis including from the interface layer of epidermis and dermis that contains regenerative basal keratinocytes; followed by stopping with 7.5 ml clinical grade Ringer’s lactate solution containing 10% of previously taken patient-own serum, for 30 s;

5) sieving the cell suspension through a 70 micrometer 50 ml Falcon tube sieve (all BD);

6) centrifugation for 7 min at 100 × g for cell washing to remove the enzymes; and
7) placing the cells into clinical grade Ringer’s lactate solution. At this stage, cell counting in a Neubauer chamber showed a yield between 0.5 and $4 \times 10^6$ cells/cm$^2$ skin, with a trypan-blue viability of the cells between 97 and 99%. Using a sterile disposable pipette (BD), the cells are transferred into sterile disposable syringes (BD) for cell deposition by spraying. We resuspended into a $1 \times 10^6$ cell/ml suspension, which we divided into three 2 ml syringes (BD) for cell spraying; C) One syringe at a time, the cell spraying was performed using fine needle spraying out of a syringe (BD) with a 30G needle (BD) through which the suspended cells are immediately spray-transplanted onto the burn site of the patient by spray deposition.

Some cells from the remaining suspension in the syringe were taken into culture, for control. The $n = 3$ culture dishes (25 cm$^2$, BD) seeded showed regular seeding and attachment behavior and regular in vitro follow up culture.

3. Results

Using skin-cell spray transplantation grafting, the “expansion” ratio of skin donor site to treatment surface area was approximately 1:20, indicating that we could cover a larger area than with a traditional graft. The wound dressing was removed on post skin cell spray-transplantation grafting day 4. The patient was noted to have complete reepithelialization of the dry wound. Wound dressings were not required anymore and the clinical picture improved with subsequent visits. No infections, inflammation or any adverse effects or complications were seen. The aesthetic and functional quality of reepithelialization at the three-, six-, and twelve-month follow-up was considered excellent. A discoloration was observed, which decreased between months three and six but had almost disappeared at month twelve. Fig. 1 shows the patient’s wounds before and after skin cell spray-transplantation. For clinical result evaluation, we used the Vancouver Scar Scale to assess the burn scar, which was 3 points after three months (2M and 1V) and 2 points (1M) after six months and 1 point after twelve months (1M).

4. Discussion

The use of enzymatically isolated [2] and in vitro expanded cells cultured to skin cell sheets [9,10] has been described to reduce mortality [11] and pain [12]. However, the take rates appear not reliable due to limitations of the method, e.g. blister formation and the loss of some skin cell populations during in vitro expansion – and the use of keratinocytes differentiated to sheets, often yields cosmetically unsatisfactory regeneration [13]. To address blister formation and subsequent sheet...
detachment, an application of single cells after expansion was introduced [14,15] which we also employed previously [16]. The concept of non-cultured cell spray-grafting enabled by immediate skin-cell preparation and application avoiding in vitro culture is based on the application of single cells sprayed onto the wound that can proliferate in the regenerating skin. Here, the cells grow to close the wounds from multiple starting points, and thus a larger ratio of donor to wound size is possible compared to conventional grafting. While providing smaller cell numbers, techniques that do not feature in vitro cell expansion appear promising, as basal keratinocytes can differentiate and lose these regenerative properties during culture [20–26]. Some authors describe a reduction of time to wound closure, which is generally known to contribute to a positive outcome [17,18]. Gravante et al., however, pointed out that mesh grafting and skin-cell spray-grafting offer comparable results and reepithelialization times and report that melanocyte grafting may not be sufficient [6]. The use of non-cultured cells was discussed controversially [19], since the cell numbers to be offered for wound regeneration are lower than with cell sheets.

Modifications of the isolation technique, as described here, can focus on the provision of cells from the basal keratinocyte layer. We employed a modified two-enzyme isolation technique that separates the dermis from the epidermis first and thus allows the subsequently used trypsin to specifically reach the regenerative keratinocytes in the epidermal part of the interface between epidermis and dermis. We also introduced an improved enzyme removal. The reproducible content and typical amount of basal keratinocytes, however, must be properly assessed in future studies.

We introduced skin-cell spray transplantation in our center to provide a therapy to a relatively rare subset of burn patients; specifically, when delayed wound healing occurs after a decision to avoid STSG. We chose an innovative practice approach since planning clinical studies in such a patient group and our limited patient population appeared challenging. Our first case on the method reported indicates that the use of sprayed autologous skin-cells in suspension can result in rapid reepithelialization and an acceptable cosmetic outcome (Fig. 1). We can confirm that a significant discoloration [5,19] occurred using single cell isolation, which should indicate that the wound was severely injured; but we saw that this reversed to almost normal cosmetic appearance between six and twelve month post grafting. Our results, however, summarize a clinical evaluation with subjective assessment of the burn depth only, a comparison to the normal healing time without the cell spray transplantation cannot be given. To give statements on whether we accelerated healing and improved cosmetic and functional outcomes, we cannot present objective markers for the assessment of the initial state of the injury. The perspective for this report was thought to be on a modified cell isolation method, easiness of the process and patient tolerance. While we have not used methods for determining more objectively if the wound healed faster than without this treatment, such as laser Doppler sonography, it was the impression of the burn surgeon that the wound would not have healed to dry, uniform epithelium by day 4 with conventional wound care only. Employing such methods, however, should be considered for clinical studies.

To conclude, we describe a novel treatment method option for second-degree burns of moderate size that do not heal in a timely manner following conservative management. The procedure could be performed in an outpatient setting, and our initial clinical data show interesting results while complications were not seen. In addition, we suspect that such a treatment may be of special interest for areas exhibiting critical wound healing such as the face or hands. Larger future clinical studies could be of interest and we are available to provide training on the method to interested centers.

**Conflict of interest**

None identified.

**Acknowledgement**

The work was sponsored by a grant from the University of Pittsburgh Medical Center, UPMC, USA.

**Author contributions**: A.C. designed, coordinated and performed the clinical work, analyzed data and wrote the manuscript. J.G. designed and coordinated the cell isolation work, developed methods for cell isolation and spraying, analyzed data and wrote the manuscript. C.J., K.B., E.M. and J.F. developed methods for cell spraying, developed methods for- and performed the cell isolation and characterization, performed laboratory analysis, analyzed data and provided discussions.

**References**


