Cell-spray auto-grafting technology for deep partial-thickness burns: Problems and solutions during clinical implementation

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Abstract

Cell-spray autografting is an innovative early treatment option for deep partial-thickness burn wounds. As an alternative to non-operative management, cell-spray autografting can achieve rapid wound re-epithelialization, particularly in large wounds. When compared to traditional mesh autografting for deep partial-thickness burn wounds, cell-spray autografting can accomplish re-epithelialization with a much smaller donor site. In this review, we describe the development of a biomedical engineering method for isolation and immediate distribution of autologous, non-cultured, adult epidermis-, and adult dermis-derived stem cells. We present data on cell isolation procedures in 44 patients with deep partial-thickness burns performed over five years under an innovative practice IRB. Treated patients presented with a variety of burn wound etiologies and a wide range of TBSA. Overall clinical results were very satisfying. The average hospital length of stay following treatment was seven days. Over the time period, the donor-site to burn-wound surface area ratio was enhanced from 1:80 to 1:100. A detailed analysis of all process-related biotechnology and operative problems, pitfalls, and solutions was performed and is reported herein. Strategies for future clinical studies are discussed.

1. Introduction

Skin transplantation has evolved from autologous full-thickness grafts to split-thickness skin grafts (STSG), to mesh expansion of STSGs into a “skin lattice” [1-3]. The most widely used technique limits the typical expansion of a STSG donor area to 1:3, as larger ratios are often associated with unsatisfactory results and complications [4-6]. The Meek technique [7] cuts the STSG into small tissue cubes, enabling a donor site to wound ratio from 1:3 to 1.9 [8]. For large burn wounds, however, the lack of available donor site remains a

Abbreviations: BMI, body mass index; DSST, donor site skin tissue; IRB, Institutional Review Board; LR, Ringer’s lactate; STSG, split-thickness skin grafts; TBSA, total body surface area.

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problem. Additional challenges exist when treating the face, joints, hands, or feet, where take rates and aesthetic outcomes are sometimes not satisfactory [9].

Deep partial-thickness burn wounds present a difficult diagnostic and prognostic challenge [10]. A conservative treatment strategy [11] of daily local wound care is often chosen by surgeons to avoid the donor site scar formation that accompanies mesh grafting [12]. However, the associated delay in re-epithelialization [13] may extend the patient’s hospitalization period, increase the risk of infection, and lead to poor functional and aesthetic outcomes, as hypertrophic scar formation can complicate delayed wound closure [14]. Early surgical intervention shortens healing time and hospital length of stay, improves functional outcomes, and limits scar formation [15].

Autologous cell-spray grafting of non-cultured epidermal cells is an innovative dermis-derived, stem cell-based therapy [16] that can be applied early using a small donor site and thus presents an alternative to conservative treatment for large deep partial-thickness burns [17]. By employing epidermal and dermal progenitor cells and using the patient’s wound as a “bioreactor” for cell expansion in situ, re-epithelialization can occur with a relatively small number of cells (as compared to STSG or cultured epidermal autografting) [18]. This method provides an on-site cell isolation process followed by the immediate application of autologous stem and progenitor cells to a freshly debrided wound bed. Cells in a saline suspension are sprayed uniformly across the wound bed to proliferate and accelerate the re-epithelialization process [17–19].

Cell-spray autografting does not preclude traditional mesh autografting, if used subsequently, and therefore could be a good early solution to address the therapeutic dilemmas attendant to deep partial-thickness burns. Furthermore, early re-epithelialization may reduce complications such as hypertrophic scarring, contracture with reduced range of motion, and poor aesthetic outcome, all of which could result in unsatisfactory psychological adjustment after therapy [20].

Our experience indicates that cell-spray autografting can enlarge the ratio of donor area to graft area from a routine 1:3 mesh to 1:100, thus facilitating operative therapy for the larger deep partial-thickness burn areas, while preserving donor area for full-thickness injuries [5]. Finally, the deposit achieved by spraying a cellular solution may improve autografting over contoured surfaces such as face and hands.

We present our experience with implementing a biomedical engineering approach in the treatment of 44 patients under innovative-practice IRB with overall very satisfactory clinical results (Table 4, online addendum). We performed a thorough analysis of all the problems that occurred; pitfalls and remedies are shared herein. Seven of the 44 patients treated were also reported previously in case reports, which include photographs of pre- and post-procedure wounds with follow-up information [17,19].

2. Material and methods

We worked as a multidisciplinary team of cell biologists, experimental surgeons, bioengineers at the University of Pittsburgh Medical Center (UPMC) Mercy Hospital. The original developer of the technology (StemCell Systems, Berlin, Germany) and an industry partner (RenovaCare, NY) were also involved to enable commercialization and marketing.

2.1. Patient criteria for cell-spray treatment inclusion

The Institutional Review Board (IRB) from UPMC Mercy Hospital, through its Technology and Innovative Practice Assessment Committee, approved the cell-based grafting procedures under an innovative practice approach. Data collection on 44 patients and retrospective analysis was performed under authorization of the Institutional Review Board (IRB # PRO14010023). Patient selection was based on the physician’s team’s best judgment but was limited to deep partial-thickness burn wounds.

2.2. Patient inclusion in cell isolation method analysis

Cell isolation was performed on 47 skin donor samples from 44 patients. Two patients had two and three cell isolation processes carried out on different days. The skin donor sample was harvested under local anesthesia using lidocaine with or without epinephrine.

2.3. Patient inclusion in wound healing studies analysis

Forty-four patients were selected for cell-spray autografting treatment of deep partial-thickness burn wounds. Forty-three patients were treated and one patient did not have enough cells to undergo treatment. Only 27 patients, however, were included in the autologous cell-spray effects and the healing time analysis section, and 11 patients with mixed partial and full-thickness burn wounds were analyzed separately. Eight patients were excluded from data analysis for a variety of reasons including poor cell recovery due to significant comorbidities (4), >80% TBSA full-thickness burn (2), and cell isolates distributed on donor sites following STSG to full-thickness wounds (2), leaving 36 patients for analysis (see Table 1).

2.4. Cell-isolation process

The process of cell isolation has been previously described and published [16–19]. The donor site skin tissue area (DSST) is calculated using the formula that combines patient’s biometric data like weight and height with the percentage of burned area to be treated. The total treatable burned area is combined with the desired seeded cell density that will be sprayed over the burn wound and divided by the constant that includes the number of isolated cells per skin square centimeter.

\[
DSST (\text{cm}^2) = \frac{0.007184 + W^{0.425} + h^{0.725} \times \text{TBSA} \cdot C_4 \times 10^2}{C_2}
\]

DSST = donor site skin tissue (cm²); W = weight (kg); h = height (cm); TBSA = total body surface area (%); C4 = cell density constant (10° cells/cm²); C2 = isolated cells per donor site skin tissue area (1,070,000 cells/cm²).
Patient split-skin donor samples of 0.2mm thickness (8/1000in.) were obtained in the burn unit by using a sterile manual dermatome (Teleflex, Limerick, PA, USA) or in the operation room with an electric dermatome (Zimmer Inc., Warsaw, IN, USA) without the use of mineral oil. After being transferred into a 100mm disposable sterile Petri-dish (Becton Dickinson, Franklin Lakes, NJ, US), the donor samples were cut into small cubes or large connected strips of skin using a surgical scalpel. The specimen was placed in a tube with 37°C pre-warmed Dispase-II solution (Roche, Indianapolis, IN, USA) for 40min to break down the dermis-epidermis connections. The epidermis was mechanically separated from the dermis using two 16G 1½in. needles (Becton Dickinson, Franklin Lakes, NJ, US). The epidermis was then digested for 15min in a 37°C pre-warmed Trypsin/EDTA-solution (0.05%/0.02%; Gibco, Life Science Technologies, Grand Island, NY, USA) with periods of intermittent shaking. Following this digestion process, two different procedures were employed to arrest the enzymatic reaction. **Method 1 (pt# 1-24):** 30ml of patient blood serum was used to stop the enzymatic reaction. The blood was incubated...
for one hour at 37°C with periods of shaking to initiate coagulation. Once the clot was formed, the blood was centrifuged for 20min at 600g/3000rpm (Ultra-8V, LW Scientific, Atlanta, GA, USA). 5-10ml of serum was carefully pipetted from the supernatant and used to stop the trypsin reaction (5ml of serum per trypsin reaction tube). **Method 2 (pt# 25-44):** The Trypsin-EDTA digestion reaction was inhibited via sequential washings with and re-suspension in Ringer’s lactate (LR) solution (the amount of sodium and calcium present in the LR is sufficient to dilute all traces of active enzyme while the initial Dis-pase-mediated digestion is effectively inhibited by the introduction of Trypsin-EDTA as the necessary magnesium and calcium required for the reaction remains and is taken up by Trypsin). Keratinocytes, sieved with a 40nm strainer (CORNING, Corning, NY, USA) are washed twice with LR (Baxter, Deerfield, IL) and centrifuged at 200g for 30s. Dermal cell isolation in the last eight patients was performed by digesting the dermal tissue with Collagenase (SERVA Electrophoresis GmbH, Heidelberg, Germany) for 40min at 37°C with periodic shaking. Dermal cell isolation was completed after centrifugation and washing in the same manner as the epidermal tissue. After the cell isolation process, the cells were mixed with the desired LR volume according to the area to spray [21].

2.5. **Wound healing measurement parameters**

After cell spray grafting, wounds were covered with standard wound dressings including Adaptic (Acclity, San Antonio, TX, USA), Mepilex silver (Mölnlycke Health Care, Gothenburg, Sweden), Biobrane (Smith & Nephew Inc., Memphis, Tennesse), N-terface (Winfield Laboratories Inc., Richardson, TX, USA), and Xeroform (Covidien PLC, Dublin, Ireland). Wound dressings were changed after three days for inspection and followed by daily changes until wound was completely re-epithelialized. Patients were deemed stable for discharge when their wounds were greater than 90% epithelialized with no signs of infection. Prior to discharge patients were assessed for the need for inpatient rehabilitation or continued outpatient physical and/or occupational therapy (Figs. 1 and 2).

2.6. **Statistics**

Kolmogorov-Smirnov student t-test for non-parametric data was performed to analyse the cell isolation time comparing the two different skin sample processing methods: cubes vs. strips (Fig. 3C). The D’Agostino & Pearson omnibus normality test showed that the measured values do not follow a normal distribution. The Pearson correlation coefficient (R²) was used for the following: burn surface area to donor site area for each of two methods, approximation and mathematical equation (Fig. 2B); isolated cell number per age and health condition (Fig. 2C); isolated cell number per donor site area for each of the two enzyme reaction methods; serum and no serum (Fig. 3F), days to discharge based on the seeded cells/burned area (Fig. 4B); and the days to discharge based on the %TBSA and the patient’s health condition (Fig. 4C). Linear regressions and correlation were performed to compare the different slopes on the effect of health conditions in Figs. 3F and 4C. All tests were performed using the statistical software package Prism vs6 GraphPath Software (La Jolla, CA, USA).

3. **Results**

3.1. **Demographic information of the treatable burn injury**

Our analysis included 47 cell isolation procedures for 44 patient treatments. The 44 patients considered exhibited deep partial-thickness burn injuries that underwent surgical debridement. Causes of burn injuries were by flame (79.55%), scalding (6.82%), grease (4.55%), chemical (4.55%), electrical (2.27%), and other etiologies (2.27%) (Fig. 1A). The analysis includes patients treated between the years 2009-2015 (Fig. 1B) with a widely different range of TBSA with treated burned areas comprising 12-5900cm² (Fig. 1B and C). No patient died due to the burn-related treatment. A comprehensive overview of the patient information is given in the on-line addendum (Table 4).

3.2. **The mathematical approach for the skin donor sample area calculations**

For the first 23 patients, the donor-site skin tissue (DSST) harvest area consideration was based on an empiric approach. The clinical demand for treating larger burn areas, however, required a more precise approach than an estimate to maximize the cell volume delivered to the wound area while maintaining a minimal DSST. We developed an equation to calculate the DSST for individual patients with a given burn wound area [22]. We calculated the DSST using this method for the follow-up group of 21 patients. We have compared these two approaches using a correlation coefficient between the burn area and the harvested DSST. For the empirical method,

![Image](image_url)
we were able to show a coefficient of determination of $R^2 = 0.47$ ($p < 0.001$) as compared to an $R^2 = 0.82$ ($p < 0.0001$) when using the mathematical approach (Fig. 2B).

3.3. **DSST thickness and the dermatome device affect cell isolation yield**

For our clinical cell-spray grafting, we used either an electrical or a manual dermatome device to harvest the DSST, depending on the operating schedule, operating room, and available equipment. The blade guards used for both devices determined the DSST thickness and were 0.03 cm (0.012 in.), 0.02 cm (0.008 in.), or 0.015 cm (0.006 in.). We found that variations in the DSST thickness generated cell isolation problems. Fig. 2A classifies the problems according to different categories. Cell clumping is defined as a cellular aggregation resulting from cells being trapped in the strainer during the isolation process. This affects the total cell yield. Enzymatic problems are defined by the inability to digest the epidermal-dermal junction or the tissue itself. Cell damage is detected through Trypan blue staining. In addition to the difficulties found during the tissue separation, patient cell number variability, health conditions, and age, may reduce the harvested cell number. The 0.02 cm (0.008 in.) thickness DSST was used most often and yielded the best results. We achieved an 80% success rate when tissue was harvested using an electrical dermatome at 0.02 cm (0.008 in.) and a 67% success rate using the manual dermatome at 0.02 cm (Fig. 2A).

3.4. **Age and health habits influence cell isolation results**

The patients’ health condition was analyzed to determine the correlation between their age and the number of cells isolated (Fig. 2C). Results were normalized based on the DSST area to allow the comparison between all patients. Along with age, two health conditions were analysed: tobacco smoking (>0.5 packet cigarettes/day) and obesity (BMI > 30). Among smokers, we saw a significant negative correlation ($p < 0.05$) between age and cell isolation numbers; 619,818 ± 552,905 (mean ± standard deviation) but no similarly significant correlation between age and cell isolation numbers among obese patients (659,763 ± 487,783). Age did not significantly impact cell isolation numbers in the normal patient group either (non-obese non-smokers) (922,091 ± 519,555) (Fig. 2C).

3.5. **Donor skin tissue ex situ procurement method and cell isolation procedure time**

The enzymatic reaction consists of an epidermal-dermal separation step with a Dispase II (Roche, Basel, Switzerland) digestion for 40 min followed by 15 min of Trypsin-EDTA
digestion for the keratinocyte isolation. Initially, the DSST was cut in small cubes of $6 \times 6$ mm (Fig. 3A) to allow more rapid penetration of the enzyme and to separate the epidermal-dermal tissues. However, with larger donor skin tissue sizes, the epidermal-dermal separation time increased substantially. Therefore, we modified the protocol to cutting long strips that remained connected at one end. Following the first enzyme digestion, effective separation could be accomplished with a single pulling action (Fig. 3B). This method showed a statistically significant reduction ($p < 0.01$) in cell isolation processing time; $94 \pm 24$ min versus $115 \pm 31$ min compared to cutting the DSST into cubes (Fig. 3C). Cutting the DSST in strips also increased the percentage of cell isolation success and decreased cell clumping and cell damage (Fig. 3D). Although the rate of enzymatic failure appeared to have increased with this
method, closer analysis of individual cases showed that this was more likely related to thicker DSST, as indicated in Fig. 2A.

3.6. Stopping the enzymatic reaction and cell isolation yield

As described in the material and method section, the enzymatic cell isolation protocol has been modified due to the problems found during the process. The early protocol included a step using patient blood serum to stop the trypsin digestion (method 1), but in some cases this triggered a clogging reaction, trapping the epidermal cells in the filter and resulting in a low cell yield (Fig. 3E). Two extra washing steps with LR solution (method 2) replaced the blood serum method. Both methods showed a significant correlation between the donor area and isolated cell number using the coefficient of determination $R^2$ (Fig. 3F). The group using serum to stop the reaction (method 1) showed a $R^2=0.31$ ($p<0.001$, $n=24$) while the group where no serum was used (method 2) showed a $R^2=0.72$ ($p<0.0001$, $n=23$). Linear regression suggests an improvement in cell yield as well with a count of 662,678 cells/cm$^2$ for method 1 compared to 932,026 cells/cm$^2$ for method 2. Eliminating the serum from the protocol resulted in 29% more cells per cm$^2$.

3.7. Cell-spray grafting outcome

Fig. 4A represents an analysis of all treated patients and the possible causes of wound healing delay separated into four groups. The normal recovery group (75%) included most of the patients with no observed complications (patients not otherwise categorized). The excluded analysis (15.9%) group includes patients that presented retrospectively with deeper wounds that underwent mesh grafting and therefore a longer hospital stay. This group also includes two patients who died as a consequence of very large TBSA (72 and 84%) full-thickness injuries, complicated by severe wound contamination and concomitant trauma, and patients with co-morbidities felt to have an influence on the overall wound healing, for example, patients with impaired circulation and/or venous stasis in the lower extremities. No patients died as a result of cell-spray grafting therapy. The pre-existing conditions group (6.8%) includes three patients required additional autografting to accelerate healing at a portion of the burn wound. No patients experienced complete failure of cell-spray autografting. There were no donor site complications. The cell isolation failure (2.3%) group includes a single patient in whom we could not obtain cells because of a blood-serum clot during enzyme-stopping (see isolation method 1) and who consequently received no spray grafting treatment.

3.8. Wound healing

The wound healing progress was analysed on deep partial-thickness burn wound patients ($n=25$), excluding those that had mixed partial and full-thickness wounds ($n=11$), or other comorbidities or mixed partial and full-thickness wounds as described in the exclusion criteria of the material and methods section. The criteria to decide if wounds were healed followed the standards described in the wound healing measuring parameters of the material and methods section.

Wound surface in this analysis included areas from 12 cm$^2$ to 9,050 cm$^2$ (1–39.5% TBSA) with an average TBSA of 15.1% ($\pm10$). Thirteen patients had TBSA $<10$; 12 had TBSA 10-19%, 7 had TBSA 20-19%, and 4 had TBSA >30%. The median cell-sprayed density was 10,555 cells/cm$^2$ wound area. Twenty-five treated patients presented partial-thickness included wound areas from 120 cm$^2$ to 5522 cm$^2$ (1 to 27% TBSA) treated with a seeded cell density median of 13,000 cells/cm$^2$ burned area (Table 2). No statistically significant correlation was found between the seeded cell density and hospital length of stay (Fig. 4B). Wound healing time was defined as the time after initial wound debridement until hospital discharge. The mean days to discharge was 6.28±4.1 with a median of six days (Table 2). When considering health habits, we showed that patients who smoke ($n=11$) underwent 5.8±5.1 hospital days, obese patients ($n=6$) underwent 8.4±4 hospital days, and non-obese/non-smokers ($n=8$) underwent 6.25±3.8 hospital days. Linear regression analysis between these groups showed no significant difference. However, there is a statistically significant positive correlation ($p<0.01$) between TBSA% and hospital length of stay, as expected, in the smoker and obese population that was not found in the “normal” group (Fig. 4C), which did not show a correlation between increasing TBSA% and hospital length of stay. Eleven patients had a combination of deep partial and full-thickness burn injuries that we treated with cell-spray grafting and traditional split-thickness
autografting (Table 3). These patients received a cell-spray treatment for burn area of <1% to 35% TBSA with a median of 12% TBSA deep partial-thickness burn wounds. Hospital length of stay for the patients in this group that survived to discharge was much longer than the simple deep partial-thickness group with a mean of 19.9 ± 18.4 days. The overall period in the hospital for some of these patients was caused by pre-existing co-morbidities (n=2) or smoke inhalation (n=4).

3.9. Evolution of the overall cell isolation procedure

Our rate of technical issues during the procedure decreased significantly over time from 65% to a current 0% technical problem rate (Fig. 5). Despite the technical difficulties listed we always were able to isolate cells and treat patients except in one case where the patient’s plasma clotted the cell suspension (Fig. 5) Substantial changes in the cell isolation protocol improved the overall cell yield and quality. The elimination of the patient plasma use for stopping the enzymatic reactions decreased the cell clumping and increased the overall cell isolation yield. The introduction of changes in the way that DSST was processed reduced the overall cell isolation time, while increasing the enzymatic permeability improving the cell yield (Fig. 5). A subsequent increase in the enzyme-treatment related failures occurred as a result of DSST harvest involving new trainees. We were able to adequately

![The Impact of Methodology on the Cell Isolation Outcome Rate](image)

Fig. 5 – Evolution of overall cell isolation outcome rate.

Cell isolation outcome percentage and the impact of methodology changes. The problems are divided into five categories defined as follows: cell clumping = excessive cell aggregation either before or after cell isolation; low cell yield = poor cell isolation; enzymatic = difficulties in epidermal-dermal separation; cell damage = cell death after the cell isolation process; success = the absence of any defined problems. The changes in the cell isolation protocol include the elimination of the serum use for stopping the enzymatic reactions, DSST processing, centrifuging step, and DSST harvesting method. Changes in the protocol for the cell isolation processes (n=47) improved the overall cell harvest over the years. Only in one patient we could not isolate enough cells for cell treatment.

| Table 2 – Summary of the burned area, seeded cell density and wound healing process for the deep partial thickness treated population (n=25). |
| Statistics | Partial thickness (TBSA%) | Seeded cells/burned area (cells/cm²) | Days to discharge (days) |
| Mean | 11.49 | 16,958 | 6.28 |
| Std. deviation | 7 | 14,515 | 4.1 |
| Median | 12 | 13,000 | 6 |
| Minimum | 1 | 1,200 | 0 |
| Maximum | 27 | 54,100 | 16 |

| Table 3 – Summary data of the treated patients combining cell-spray therapy with split-thickness skin graft. The treated patients showed different partial and full-thickness TBSA% (n=11). |
| Statistics | Partial thickness (TBSA%) | Full thickness (TBSA%) | Days to discharge (days) |
| Mean | 14.3 | 6.88 | 19.91 |
| Std. deviation | 11 | 6.44 | 18.41 |
| Median | 11.9 | 4 | 11 |
| Minimum | 0.55 | 1.2 | 5 |
| Maximum | 35 | 21.4 | 58 |
address the issue by identifying an education gap when training new technical assistants. The changes in the DSST harvesting method reduced the percentage of cell isolation failures to 0%. Other modifications in the cell isolation methodology, such as reducing the centrifugation speed, at 200g from 5 min to 30s, helped to minimize the cell damage and enhance the cell yield.

4. Discussion

The initial work was focused on the cell isolation and cell spray-grafting as case report data collection. As therapy quality control we included comprehensive documentation of the treatments, outcome and recovery process for subsequent data analysis. The initial goal of this study analysis was looking into the clinical records of all the treated patients with skin cell spray grafting since June 2009. The collection of relevant variables included patient characteristics and comorbidities, burn characteristics, details of the particular cell spray graft procedure, treatments used in addition to cell spray grafting, and functional and aesthetic outcomes of the treatment. The data analyses allowed us to identify factors that are associated with favorable or unfavorable outcomes, creating leads for potential future clinical studies and improvements in the cell spray grafting protocol.

Various skin cell isolation protocols have been previously described [23,24] but detailed practical information on methodology, pitfalls, reproducibility and reliability is lacking. We presented all technical issues identified during the clinical implementation of the procedure to enable burn surgeons to learn from our experience.

4.1. Patient selection and limitations in donor area size

The cell-spray technique was mainly used to treat flame burn injuries, but not only and exclusively (Fig. 1A). Starting in 2012, we developed a routine for such treatments (Fig. 1B). The treatable burn area covered a broad range up to 7000 cm², with the majority between 1000 and 3000 cm² (Fig. 1B and C). Other similar available techniques, such as the ReCell kit (Avita, South Perth, Australia), were developed for isolating cells from a 4 cm² DSST area, while their practice is limited to 320 cm² burn area (91% of our treated patients exhibited larger burn surfaces). On the other hand, cultured epidermal autograft (CEA) technology, such as Epicell (Vericell, Cambridge, MA, USA) [25], uses a small amount of patient skin DSST to regenerate burn areas greater than 30%. However, because this technology uses mouse cells lines, the FDA limits its use to treatment in patients with >30% TBSA as a Humanitarian Device Exemption [26]. This limitation, in addition to the long wait time (between 16-21 days), cost, and graft fragility [27], makes the procedure unsuitable for most patients. Autologous cell-spray grafting has the advantages of being immediately available, requiring minimal cell manipulation, using the patient’s body as a “bioreactor” for cell expansion, and limiting the loss of progenitor cells and epidermal stem cells [16].

4.2. Therapy planning

Minimizing time in the operating room as well as limiting the time under general anesthesia is important for patients, medical staff, and operating costs. Before harvesting of the DSST we used a mathematical formula to streamline the process of harvesting the minimum amount of donor skin necessary to cover the burn wound area reaching a 1:100 ratio [21]. The entire cell isolation process, which includes the DSST harvest, takes approximately two hours and can be performed by a nurse or trained technician. To reduce operation room expenses in those patients where the wounds did not undergo extensive wound preparation, we took the DSST under local anesthesia around two hours before the patient enters the operation room. After the cell isolation protocol, the cells were diluted with the Lactated Ringers solution and stored until needed at 4 °C under sterile conditions in a labeled container. We concluded that in situations where also mesh grafting is planned, the cell isolation process could be performed at the same time as the initiation of anesthesia, debridement, and mesh grafting took place. We quickly changed our practice to obtaining the donor skin under local anesthesia and sterile conditions in our burn unit procedure suite prior to patients’ entering the OR and undergoing general anesthesia [19].

4.3. Modifications in the cell isolation method

We found that DSST thickness is critical to optimizing cell isolation. We obtained the best results using an electrical dermatome at a 0.02 cm (8/1000in.) guard setting (Fig. 2). Using a manual dermatome (Weck blade with 0.008 in. guard) resulted in greater variability and even led to a provider-related technical issue when the thickness of the DSST led to enzyme failure (Fig. 5), requiring education and re-training. A thicker DSST can lead to problems separating the epidermal-dermal junction during the Dispace reaction, likely because of the difficulty of penetrating into the epidermal-dermal junction. We also found that cell clumping, low cell yield, and enzymatic problems were associated with the increased DSST thickness. To address the problem, we considered using a 0.015 cm blade guard instead. However, when 0.015 cm guard blades are applied correctly on the skin surface, they can generate too thin a tissue sample, excluding the epidermal stratum basale, where the epidermal stem cells and proliferative progenitor cells reside. We concluded that staff training and donor skin tissue thickness play an important role in cell isolation with the optimal result being an electric dermatome set at 0.02 cm or a manual dermatome with a similar guard in the hands of an experienced technician. As discussed above, the time needed for donor area skin processing and cell isolation is important. Cutting the skin donor sample in strips instead of into squares (Fig. 3A and B) reduced the time for the procedure (Fig. 3C), increased the percentage of cell isolation success, and decreased cell clumping and cell damage (Fig. 3D and Fig. 5).

Results (Fig. 5) suggest that the rate of enzymatic failures increased when we stopped using patient’s plasma, but individual examination of cases showed that this issue was related to a thicker DSST (as previously described). Traditionally, serum is added to cell cultures to stop the enzymatic action. We initially used autologous, heparinized serum for
this step but found several issues with cell clumping and low cell yields. Replacing the use of serum with LR washings followed by centrifugation resulted in 29% more cells per cm² DSST area (Fig. 3E). This change also had the advantage of avoiding the additional procedural steps of blood draw and centrifugation.

4.4. The influence of age and health habits on outcomes

The outcome procedure analysis did not show significant differences when the days of hospital discharge after procedure or the number of sprayed cells per burned area were correlated (Fig. 4B). While a paucity of isolated cells, due to a less than optimal isolation procedure, may result in slower wound healing, it does not appear that an excess of cells necessarily results in an acceleration of wound healing as measured by hospital length of stay.

As expected, patients with large burn areas received a longer hospital stay after procedure (Fig. 4C). When comparing hospital length of stay after procedure with % TBSA of the wounds treated comparing patients with smoking habits, or obesity, we found a significant correlation between tobacco smoking habits (p<0.001) and an increase of the hospital length of stay. Although no relationship between %TBSA and hospital length of stay was seen obesity group or the “normal” (non-obese, non-smokers) group, further analysis is required to confirm this correlation and discard any artificial interference.

On the other hand, our data does show a correlation between age and cell isolation outcome (Fig. 2C), confirming previous results that age and health habits have an impact on cell attachment and survival [28]. We suggest that these aspects may be considered when planning clinical studies, as individual patient factors may play a role and could have an impact on meeting study endpoints.

A control group with no cell-spray treatment would be of interest for further comparisons on the effect of seeded cell densities as well as the influence of various health conditions on outcomes. Furthermore, we recognize that hospital length of stay has significant limitations as a marker for wound re-epithelialization. This is an important metric that needs to be better defined and studied in the future, especially for clinical trials.

Our findings may provide valuable information for planning future clinical studies in the area. Staff training, appropriate quality control, and quality management was critical for method implementation. The potential effect on the outcomes resulting from slightest change in the procedure should be specifically monitored and if possible tested in advance in in vitro settings.

Conflict of Interest

J. G. has a potential conflict of interest through a partial ownership with StemCell Systems, Berlin, Germany and royalties from RenovaCare, NY, that are both active in this area of research. The University of Pittsburgh manages his conflict of interest in accordance with its policies and procedures.

Author contributions

R.E and J.G wrote the manuscript. R.E and M.C compiled, and analyzed the data. M.Y. performed the cell isolation. A.C., J.Z., and M.C revised the manuscript and provided discussions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.burns.2017.10.008.

References


